Endothelial dysfunction and arterial pressure regulation during early diabetes in mice: roles for nitric oxide and endothelium-derived hyperpolarizing factor


Departments of 1Physiology and 3Pharmacology, Monash University, and 3Neuropharmacology Laboratory, Baker Heart Research Institute, Melbourne, Australia

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Fitzgerald SM, Kemp-Harper BK, Parkington HC, Head GA, Evans RG. Endothelial dysfunction and arterial pressure regulation during early diabetes in mice: roles for nitric oxide and endothelium-derived hyperpolarizing factor. Am J Physiol Regul Integr Comp Physiol 293: R707–R713, 2007. First published May 23, 2007; doi:10.1152/ajpregu.00807.2006.—We determined whether nitric oxide (NO) counters the development of hypertension at the onset of diabetes in mice, whether this is dependent on endothelial NO synthase (eNOS), and whether non-NO endothelium-dependent vasodilator mechanisms are altered in diabetes in mice. Male mice were instrumented for chronic measurement of mean arterial pressure (MAP). In wild-type mice, MAP was greater after 5 wk of Nω-nitro-L-arginine methyl ester (L-NAME); 100 mg·kg⁻¹·day⁻¹ in drinking water; 97 ± 3 mmHg) than after vehicle treatment (88 ± 3 mmHg). MAP was also elevated in eNOS null mice (113 ± 4 mmHg). Seven days after streptozotocin treatment (200 mg/kg iv) MAP was further increased in L-NAME-treated mice (108 ± 5 mmHg) but not in vehicle-treated mice (88 ± 3 mmHg) nor eNOS null mice (104 ± 3 mmHg). In wild-type mice, maximal vasorelaxation of mesenteric arteries from wild-type mice with a targeted deletion of the endothelial isoform of NOS (eNOS) was reduced by 42 ± 6% in L-NAME-treated diabetic mice. Furthermore, the relative roles of NO and endothelium-derived hyperpolarizing factor (EDHF) in acetylcholine-induced vasorelaxation were altered; the EDHF component was enhanced by L-NAME and blunted by diabetes. These data suggest that NO protects against the development of hypertension during early-stage diabetes in mice, even in the absence of eNOS. Furthermore, in mesenteric arteries, diabetes is associated with reduced EDHF function, with an apparent compensatory increase in NO function. Thus, prior inhibition of NOS results in endothelial dysfunction in early diabetes, since the diabetes-induced reduction in EDHF function cannot be compensated by increases in NO production.

While in established diabetes reduced NO synthesis and enhanced NO breakdown likely make an important contribution to endothelium-dependent vasodilator dysfunction (36), there is also evidence that NO production is enhanced in the early stages of diabetes in some organs (18). Consistent with this view, induction of diabetes led to the development of hypertension in rats pretreated with the nonspecific NO synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (L-NAME), but not in rats with intact NOS (14, 15). Thus, at least in rats, NO appears to protect against the development of hypertension in the early stages of diabetes. This protective influence of NO on arterial pressure appears to result from its actions to buffer diabetes-induced pressor mechanisms. Hence, removal of the influence of NO allows these pressor effects to dominate, resulting in hypertension. In addition, there is good evidence that endothelial function, particularly that mediated by EDHF, is blunted in diabetes (22, 27, 37). Therefore, in the present study we examined the relationship between diabetes-induced changes in the vascular endothelium and control of arterial pressure at the onset of diabetes.

Our experiments were performed in mice to provide an avenue for analysis of the possible genetic factors governing the cardiovascular responses to induction of diabetes. We tested responses of 24-h mean arterial pressure (MAP) and heart rate (HR) to induction of diabetes in wild-type mice, wild-type mice with hypertension induced by 5-wk treatment with L-NAME (100 mg·kg⁻¹·day⁻¹ in drinking water), and mice with a targeted deletion of the endothelial isoform of NOS (eNOS −/−). Thus, we were able to determine whether NO protects against the development of hypertension during the early stages of diabetes in mice as in rats and whether this is dependent on eNOS. Responses to acetylcholine and the NO donor diethylamine NONOate (DEA/NO) were then examined in isolated mesenteric arteries from wild-type mice treated chronically with L-NAME or its vehicle (5 wk in drinking water) with and without induction of diabetes. This allowed us to characterize the effects of, and interactions between, diabetes and chronic NOS inhibition on the relative contributions of NO, prostanoids, and EDHF to endothelium-dependent vasodilation in the mesenteric vasculature.

METHODS

Animals. Experiments were performed on a total of 33 C57BL/6J male mice (12-wk-old; 30 ± 1 g when entering the experiment) and 16 eNOS −/− mice (30 ± 1 g) obtained from breeding colonies at

Address for reprint requests and other correspondence: R. G. Evans, Dept. of Physiology, PO Box 13F, Monash Univ., Victoria 3800, Australia (e-mail: roger.evans@med.monash.edu.au).

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Monash University MouseWorks Facility. The eNOS−/− mouse colony was initially established with founders from Jackson Laboratory (Bar Harbor, ME) originally derived by Shesely et al. (35). During the experimental period, mice were housed in a room with a 12:12-h light-dark cycle (0700 AM–0700 PM), relative humidity of 50 ± 2% and temperature of 21 ± 1°C. The mice received normal rodent chow and tap water ad libitum throughout the study. All animal procedures were approved in advance by the Monash University, Department of Physiology Animal Ethics Committee and were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Surgery for catheter implantation. Mice were anesthetized with isoflurane (Forthane; 4–5% vol/vol induction and 1.5–2.0% vol/vol maintenance; Abbott Australasia, Kurnell, NSW, Australia). Body temperature was maintained at ~37°C by using a heating pad. With the use of aseptic techniques, catheters (0.58 mm ID, 0.96 mm OD, drawn over heat; SIMS Portex, Keene, NH) were placed 1) in the carotid artery for direct measurement of MAP and HR and 2) in the jugular vein for drug administration. A similar technique has been successfully used to measure both acute and chronic changes in MAP and HR following various drug regimens in the conscious mouse (23, 24). The catheters were tunneled subcutaneously, exteriorized, and passed through a stainless steel button, which was then secured in place between the scapulae. Mice were allowed to recover from anesthesia before being transferred to the experimental room. The button was connected via a guidewire to a counterbalance arm, to which a dual-channel swivel was attached (AgnThos, Lidingö, Sweden).

Both catheters were connected to infusion pumps set to deliver either heparinized saline (27 IU/ml; 0.39 μl/min; via the carotid artery) or saline (0.9% wt/vol NaCl, 0.6 μl/min; via the jugular vein) to maintain catheter patency and to allow undisturbed drug administration. Pulsatile arterial pressure was digitized (using a program written in LabView; National Instruments, Austin, TX) and recorded continuously for up to 15 days. Hemodynamic measurements recorded included systolic, diastolic, and calculated MAP and HR (17, 28). Data were analyzed by using a specifically written data analysis and fitting program (CIRCAD, version 2003; Dr. G. A. Head, Baker Heart Research Institute, Victoria, Australia). Using this data analysis program, we could assess circadian rhythm by calculating the greatest level of MAP observed during the active night phase (peak) and the lowest level of MAP observed during the quiet day phase (nadir).

Protocol 1: effects of chronic l-NAME or eNOS gene deletion, on the hemodynamic responses to 7 days of diabetes. In this experiment, we tested MAP and HR responses, over the first 7 days after induction of diabetes with streptozotocin (STZ) in wild-type C57BL/6J mice treated chronically with the NOS inhibitor l-NAME or its vehicle and in eNOS−/− mice. STZ or its vehicle was administered as a single dose [200 mg/kg iv in 8 ml/kg of sodium citrate buffer (pH 4.5)] after 4 wk of l-NAME or its vehicle. Thus, six groups of mice were studied: 1) wild-type vehicle treated (plain drinking water; n = 11), 2) wild-type diabetic (n = 10), 3) wild-type l-NAME treated (100 mg·kg·1·day−1 in drinking water for 5 wk; n = 6), 4) wild-type l-NAME-treated diabetic (n = 6), 5) eNOS−/− (n = 7), and 6) eNOS−/− diabetic (n = 9). MAP and HR responses were measured continuously for 7 days after STZ or its vehicle were administered. Because STZ or its vehicle were administered after 4 wk of treatment with l-NAME or its vehicle, at the conclusion of the experiment l-NAME-treated mice had received a full 5 wk of treatment with l-NAME. At the completion of the experiment, mesenteric arteries were removed for the functional studies outlined in protocol 2. At this point, a blood sample was also taken for assessment of blood glucose concentration. Mice in which blood glucose levels were > 250 mg/dl were considered diabetic.

Protocol 2: effects of chronic l-NAME on diabetes-induced changes in endothelium-dependent relaxation of isolated mesenteric arteries. Functional studies were performed in small mesenteric arteries taken from a cohort of male wild-type mice from the groups outlined above at the completion of the experiments: 1) vehicle treated (n = 4–5), 2) diabetic (n = 4–6), 3) l-NAME treated (n = 4), and 4) l-NAME treated + diabetic (n = 4). Mice were killed via cervical dislocation and exsanguination, and second-order mesenteric arteries were isolated, cut into 2-mm lengths, and mounted in isometric myographs (26). Vessels were maintained in physiological Krebs solution (composition in mm: 119 NaCl, 4.7 KCl, 1.17 MgSO4, 25 NaHCO3, 1.18 KH2PO4, 2.5 CaCl2, 11.1 glucose, and EDTA 0.026) at 37°C and bubbled continuously with 95% O2-5% CO2. Data were captured via a computerized vascular monitoring system data acquisition system (World Precision Instruments, Sarasota, FL). Following a 30-min equilibration period, vessel diameters were normalized to an equivalent transmural pressure of 100 mmHg (26).

To elucidate the relative contribution of vasodilator prostanooids, NO, and EDHF to acetylcholine-mediated vasorelaxation, responses to acetylcholine were obtained in the absence and presence of 1) the cyclooxygenase inhibitor indomethacin (3 μM); 2) indomethacin (3 μM) in combination with l-NAME (100 μM) and an inhibitor of the NO receptor, soluble guanylate cyclase, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ: 10 μM); 3) indomethacin (3 μM) in combination with charybdotoxin (50 nM), an inhibitor of large and intermediate conductance Ca2+-activated potassium channels (KCa); and apamin (100 nM), an inhibitor of small conductance KCa; and 4) indomethacin (3 μM) in combination with l-NAME (100 μM), ODQ (10 μM), charybdotoxin (50 nM), and apamin (100 nM). ODQ was used to ensure that the effects of any residual NO following chronic NOS inhibition (e.g., from preformed stores) were removed. At the completion of each concentration response curve, maximal relaxation was established by the combined addition of the NO donor sodium nitroprusside (10 μM) and the ATP-sensitive potassium channel opener levcromakalim (10 μM).

Statistical methods. Data are expressed as means ± SE and two-tailed P ≤ 0.05 was accepted as statistically significant. For the in vivo study (protocol 1), within-group and between-group differences were determined using ANOVA with post hoc Bonferroni tests and where appropriate, paired- and unpaired t-tests (Systat version 9; SPSS, Chicago, IL) (20). For in vitro studies (protocol 2), relaxation responses were expressed as the percentage reversal of U-46619 precontraction. Individual relaxation curves were fitted to a sigmoidal logistic equation (Prism version 3.0; Graphpad, San Diego, CA) and concentration of agonist giving a 50% relaxation (pEC50) values calculated and expressed as −logM. The statistical significance of between-treatment differences in mean pEC50 and maximum relaxation (Rmax) values were tested using a one-way ANOVA followed, where appropriate, with a Dunnett’s modified t-test (Graphpad Prism) (20). Where a maximum response was not obtained, and thus pEC50 values could not be determined, two-way ANOVA was used to compare concentration response curves (version 1.0; SigmaStat, San Jose, CA). The post-ANOVA Student-Newman-Keuls test was used as appropriate.

Materials. Drugs and their sources were: ODQ and U-46619 (Sapphire Bioscience, Crows Nest, NSW, Australia); acetylcholine,
RESULTS

Protocol 1: basal MAP and HR after chronic 1-NAME treatment and in eNOS −/− mice and the hemodynamic responses to 7 days of diabetes. Following chronic 1-NAME treatment (100 mg·kg−1·day−1, orally) wild-type mice had a significantly greater MAP (97 ± 3 mmHg) than vehicle-treated mice (88 ± 3 mmHg, P ≤ 0.05). MAP in eNOS −/− mice was significantly greater (113 ± 4 mmHg, P ≤ 0.05) than either vehicle-treated or 1-NAME-treated wild-type mice (Fig. 1). HR was indistinguishable in the three groups of mice. Induction of diabetes did not significantly affect MAP in vehicle-treated wild-type mice (88 ± 3 mmHg), eNOS −/− mice (104 ± 3 mmHg), but was associated with a further elevation of MAP in mice treated chronically with 1-NAME (to 108 ± 5 mmHg, 7 days after STZ administration). Also of interest was the finding that the day/night difference in MAP was significantly reduced in the 24 h immediately following administration of STZ. This effect was observed in both 1-NAME-treated and vehicle-treated wild-type mice and in eNOS −/− mice. Before STZ, the day/night MAP difference was 11 ± 2 mmHg, but in the 24 h after STZ it was 0 ± 1 mmHg (averaged across all STZ-treated mice, P ≤ 0.001). The day/night MAP difference slowly returned to its control level over the next 3–4 days. By day 7 after STZ administration it did not differ significantly from its control level (13 ± 2 mmHg). HR was significantly less in STZ-treated diabetic mice compared with mice treated with vehicle. The magnitude of this effect was similar in wild-type mice (1-NAME and vehicle-treated) and eNOS −/− (diabetic: 396 ± 11 beats/min averaged across all three diabetic groups vs. nondiabetic: 482 ± 13 beats/min; Fig. 1).

Seven days after STZ-treatment, blood glucose concentrations were 400 ± 25, 399 ± 42, and 419 ± 29 mg/dl, respectively, in vehicle-treated wild-type mice, 1-NAME treated wild-type mice, and eNOS −/− mice. These blood glucose levels were approximately threefold greater than in corresponding mice receiving the vehicle for STZ (149 ± 11, 154 ± 21, and 158 ± 17 mg/dl, respectively).

Protocol 2: effects and interactions of chronic 1-NAME and diabetes on endothelium-dependent and -independent relaxation of isolated mesenteric arteries. Acetylcholine caused concentration-dependent relaxation of small mesenteric arteries from vehicle-treated wild-type mice (pEC50 = 7.00 ± 0.29, Rmax = 88 ± 3%; Fig. 2A, n = 4). Neither the maximal response to acetylcholine, nor its potency, was altered by chronic 1-NAME treatment or induction of diabetes (Table 1). In contrast, the maximum response to acetylcholine was reduced to 51 ± 6% (P ≤ 0.01) in mice in which diabetes was induced following chronic 1-NAME treatment (Fig. 2A). The pEC50 (6.76 ± 0.28) for acetylcholine, in vessels from mice treated with both 1-NAME and STZ, did not differ significantly from that in vessels from vehicle-treated mice. The NO donor, DEA/NO relaxed mesenteric arteries from vehicle-treated animals by 93 ± 4% (pEC50 = 7.04 ± 0.20, n = 4). This response was similar in vessels taken from mice treated with 1-NAME, STZ, or 1-NAME in combination with STZ (Fig. 2B).
response to acetylcholine was markedly attenuated in the presence of charybdotoxin and apamin in combination with L-NAME and ODQ (~60-fold decrease in sensitivity $P \leq 0.05$; $R_{max} = 40 \pm 12\%$, $P \leq 0.01$, Fig. 3A).

Following chronic treatment with L-NAME (100 mg·kg$^{-1}$·day$^{-1}$), vasorelaxation to acetylcholine ($pEC_{50} = 6.96 \pm 0.21$, $R_{max} = 87 \pm 7\%$) was unaffected by indomethacin. However, the sensitivity to acetylcholine was decreased ~40-fold in the presence of L-NAME, ODQ, and indomethacin ($P \leq 0.05$ vs. control response; Fig. 3B, Table 1). The combination of indomethacin, charybdotoxin and apamin virtually abolished the response to acetylcholine (relaxation to 10 μM acetylcholine: 6 ± 4%, $P \leq 0.01$ vs. 87 ± 7% in control).

In small mesenteric arteries from diabetic mice, vasorelaxation to acetylcholine ($pEC_{50} = 7.19 \pm 0.43$, $R_{max} = 94 \pm 2\%$) was not significantly altered by indomethacin, L-NAME, and ODQ in combination or charybdotoxin + apamin (Fig. 3C). However, the combination of indomethacin, L-NAME, ODQ, charybdotoxin, and apamin completely abolished the response to acetylcholine (relaxation to 10 μM acetylcholine: 5 ± 3%, $P \leq 0.01$ vs. control response, Fig. 3C, Table 1).

In vessels from diabetic mice treated chronically with L-NAME, neither indomethacin alone nor L-NAME and ODQ in combination with indomethacin significantly affected the relaxation response to acetylcholine (Fig. 3D, Table 1). In contrast, charybdotoxin, apamin, and indomethacin virtually abolished vasorelaxation to acetylcholine (relaxation at 10 μM acetylcholine: 11 ± 7%, $P \leq 0.05$ vs. vehicle). Responses to acetylcholine were also virtually abolished after treatment with indomethacin, charybdotoxin, apamin, L-NAME, and ODQ (Fig. 3D, Table 1).

**DISCUSSION**

In the present study, we found that under conditions of intact NOS, diabetes did not significantly alter arterial pressure in mice. Rather, a pressor response to the induction of diabetes was only observed following chronic pharmacological NOS inhibition, as has been shown previously in rats (14, 15). Similarly, at the level of the mesenteric vasculature, diabetes was only associated with an impairment of endothelium-dependent vasorelaxation following chronic NOS inhibition. Thus, our present studies demonstrate that diabetes-induced endothelial dysfunction and hypertension is only revealed following pharmacological inhibition of NOS. Interestingly, diabetes did not further increase arterial pressure in eNOS$^{-/-}$ mice, indicating that eNOS is not an absolute requirement for the cardiovascular adjustments that normally prevent development of hypertension in early diabetes.

We used two different mouse models to elucidate the role of NO in the cardiovascular adjustments to diabetes. First, mice were treated chronically with the nonselective NOS inhibitor, L-NAME. To delve further into the role of specific NOS isoforms, we utilized eNOS$^{-/-}$ mice. Chronic (5-wk)
L-NAME treatment via drinking water significantly increased MAP. Consistent with our present findings, Chatziantoniou et al. (7) found that in mice, chronic L-NAME treatment produced a gradual increase in arterial pressure (measured by tail cuff) over a period of 14 wk. Other studies have also demonstrated pressor responses to L-NAME, administered via the drinking water, of comparable magnitude to that which we observed in the present study (2, 29, 30, 32). The fact that MAP was lower in mice treated chronically with L-NAME than in eNOS⁻/⁻ mice may reflect incomplete blockade of NOS by this oral L-NAME treatment. Nevertheless, the level of NOS blockade induced by L-NAME was sufficient to uncover an underlying pressor response to diabetes.

In our present study, arterial pressure was measured continuously in unanesthetized mice. Furthermore, our experimental measurements were made at least 6 days after surgery, since circadian rhythms of MAP and HR did not stabilize until ~5 days postsurgery. Hence, our present observations were not confounded by the acute effects of anesthesia nor by carryover effects of the surgical procedure on cardiovascular control.

Based on the findings from our chronic L-NAME mouse model, it is clear that NO counterregulates the underlying pressor response in early-stage diabetes, consistent with previous findings in rats (14, 15). To elucidate the potential source of this protective NO, diabetes was also induced in eNOS⁻/⁻ mice, which are hypertensive under resting conditions. The

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**Fig. 3.** Effects of 7 days of diabetes on the relative contributions of vasodilator prostanoids, nitric oxide, and endothelium-derived hyperpolarizing factor to endothelium-dependent relaxation. Concentration response curves to acetylcholine (ACh) were evaluated in small mesenteric arteries taken from C57BL/6J mice following treatment with either vehicle (A; n = 4–5), L-NAME, (B; 100 mg·kg⁻¹·day⁻¹ for 5 wk; n = 4), STZ (C; 200 mg/kg single dose 7 days prior to the experiment; n = 4–6) or L-NAME + STZ (D; n = 4). In groups (A–D) responses were obtained under control conditions and in the presence of indomethacin (Indo; 3 μM); Indo (3 μM) + L-NAME (100 μM); and inH-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ; 10 μM). Values are expressed as %reversal of precontraction and given as means ± SE, where n = the number of vessel segments studied. *P ≤ 0.05 for treatment concentration response curves vs. control (2-way ANOVA, Student-Newman-Keuls test). #P ≤ 0.05, ##P ≤ 0.01 for responses at 10 μM acetylcholine vs. control (1-way ANOVA, Dunnett’s modified t-test).
induction of diabetes in these mice did not result in further increases in arterial pressure. These findings could be interpreted to suggest that eNOS is not the source of protective NO in early-stage diabetes. Given that L-NAME is a nonselective NOS inhibitor, other NOS isoforms, namely neuronal NOS (nNOS) and/or inducible NOS (iNOS), may play a role in generating the NO that is antihypertensive in early diabetes. Alternatively, the absence of a pressor response to induction of diabetes in eNOS −/− mice might reflect compensatory up-regulation of other NOS isoforms and/or other endothelial-derived vasodilator factors in these mutant animals. Resolution of these issues will not be straightforward, given the potential pitfalls associated with the use of so-called selective inhibitors of NOS isoforms and mice with targeted deletion of NOS isoforms and the difficulties associated with quantifying the contributions of various endothelial factors to the control of arterial pressure under physiological conditions.

A further aim of this study was to investigate the effects of diabetes on endothelial function at the level of the vasculature using the well-studied mesenteric artery. In nondiabetic mice, endothelium-dependent relaxation in small mesenteric arteries appeared to be mediated predominantly by EDHF, with NO serving as a back-up vasodilator pathway in its absence. These findings are consistent with those of previous studies in mouse mesenteric arteries (11, 21) although other observations suggest a more prominent role for NO (6, 10). Such discrepancies may reflect differences in the age of the mice, the size of the mesenteric arteries studied, and/or the level of resting tension applied to the vessel.

Consistent with previous in vitro studies of endothelial function (27, 34), diabetes alone did not impair acetylcholine-induced endothelium-dependent vasorelaxation in mesenteric resistance arteries. Nevertheless, diabetes diminished the contribution of EDHF to endothelium-dependent relaxation, while leading to an apparent compensatory increase in the contribution of NO. These findings are in agreement with previous observations of endothelial-dependent vasodilation in mesenteric arteries from mice with type 1 diabetes (22, 27). These observations suggest a potential compensatory protective role of NO in the resistance vasculature at least in early diabetes, which concurs with our in vivo observations with regard to arterial pressure control. Of note, the source of NO conferring protection may differ across a range of vascular beds in the intact animal.

Significant impairment of endothelium-dependent vasorelaxation per se was only observed when diabetes was induced on a background of chronic NOS inhibition. The impaired response to acetylcholine under these conditions does not reflect an inability of the vascular smooth muscle to relax, as the response to the NO donor DEA/NO was similar in vessels from all groups of mice studied. Rather, such endothelial dysfunction arose as a consequence of a diabetes-induced decrease in the contribution of EDHF to vasorelaxation together with a loss of compensatory NO.

The mechanisms underlying impairment of EDHF-mediated vasodilation in type 1 diabetes remain to be elucidated. Dysfunction may occur at the level of EDHF synthesis or release, gap junction integrity, transduction, and/or potassium channel modulation. It has recently been proposed that lysophosphatidylcholine, which is released from oxidized low-density lipoproteins and elevated in diabetes, may impair EDHF production or release in diabetes by increasing production of superoxide in the vasculature (22). An alternative mechanism may involve myoendothelial gap junctions, which are composed of connexin proteins and appear to be crucial for the EDHF response in mouse small mesenteric arteries (11). However, the effects of diabetes on expression of connexin peptides remain a matter of controversy (9, 22).

Of interest was the finding that chronic NOS inhibition with L-NAME enhanced the contribution of EDHF to endothelium-dependent relaxation. Indeed, NO can inhibit EDHF function (1) and upregulation of EDHF has previously been observed in the vasculature of eNOS −/− mice (10, 33). Surprisingly, acetylcholine-induced vasorelaxation of vessels from mice treated chronically with L-NAME was attenuated following acute treatment with L-NAME, the soluble guanylate cyclase inhibitor ODQ, and indomethacin, but not indomethacin alone. This suggests that chronic L-NAME treatment may not completely abolish NO synthesis, as has been previously observed with chronic administration of N^ω-nitro-L-arginine (19). If NOS inhibition was incomplete under the conditions of our experiment, the residual source of NO appears to be lost upon induction of diabetes, since we found that acetylcholine-induced vasorelaxation was unaffected by L-NAME + ODQ in mesenteric arteries from diabetic mice chronically treated with L-NAME. However, we were unable to directly assess the extent of NOS blockade in vivo, so such conclusions must be viewed with caution.

Our results suggest that both diabetes and chronic L-NAME treatment alter the relative contributions of NO and EDHF to endothelium-dependent vasodilation in mesenteric arteries. Early-stage diabetes appears to reduce EDHF function, but the subsequent loss of vasodilator reserve appears to be compensated for by NO. In addition, chronic NOS inhibition appears to increase the dependency of endothelium-dependent vasodilation in mesenteric vessels on EDHF. Conversely, the induction of diabetes in the setting of NOS inhibition attenuates EDHF-mediated responses and impairs endothelium-dependent vasorelaxation. Upon initial consideration, these in vitro findings appear to correlate well with our in vivo observations such that a significant impairment in the response to acetylcholine in mesenteric arteries and elevation in arterial pressure was only observed by the combined effect of diabetes and chronic NOS inhibition. However, in the mesenteric bed at least, the source of compensatory NO is presumably from eNOS, while in the intact mouse the compensatory adjustments that prevent hypertension at the onset of diabetes can occur in the absence of eNOS.

In conclusion, our present observations indicate that NO is crucial in countering the development of hypertension during the early stages of type 1 diabetes in mice, as has previously been demonstrated in rats (14, 15). Our studies of endothelium-dependent vasodilation in mesenteric arteries in vitro are consistent with the hypothesis that diabetes reduces EDHF function, yet such changes are unlikely to reflect the entire spectrum of events influencing arterial pressure in the intact animal. Given hypertension did not develop after the induction of diabetes in eNOS −/− mice, we hypothesize that diabetes may upregulate other NOS isoforms in a compensatory manner, such as nNOS (3, 4, 18) or iNOS, in view of the proinflammatory events associated with diabetes. Thus NO appears to be a crucial counterregulatory factor that prevents development of...
hypertension during the early stages of diabetes, but the source of this NO remains to be determined.

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Present address of S. M. Fitzgerald: Population Health Research Unit, Baker Heart Research Institute, PO Box 6492, St. Kilda Road Central, Melbourne, VIC 8008, Australia.

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